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Molecular Dynamics Studies of the RNA-Dependent RNA Polymerase of the Hepatitis C Virus

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The Hepatitis C Virus (HCV) affects approximately 200 million people throughout the world. About 25% of individuals with HCV will eventually contract chronic liver ailments, such as cirrhosis or liver cancer. Currently, there is no cure for this disease and there are few effective treatments. The HCV RNA-dependent RNA polymerase (RdRp) is an enzyme that is presently a target protein for drug discovery because of its importance for viral replication. It is believed that a conformational change is necessary for RdRp to initiate the replication process. There is evidence that this conformational change is facilitated by the presence of magnesium ions and crystallographic data indicates the location of two magnesium-binding sites in the palm domain of the enzyme. We employ molecular simulations and Principal Component Analysis to demonstrate, at a molecular level of detail, that the presence of magnesium ions alters the internal motion of RdRp. Particularly large fluctuations observed in the thumb domain of the enzyme may play a role in mediating the conformational change necessary for RdRp activity. By observing the structural coupling that occurs as a result of enzyme dynamics, we hope to understand the link between the dynamic properties of RdRp and its functional attributes. In addition to providing insight into RdRp function, this study illuminates fundamental questions regarding the role that external effectors may play in altering internal enzyme dynamics.

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Trapped in Cristae: Localization and Tracking of Mitochondrial Membrane-Proteins in Living Cells

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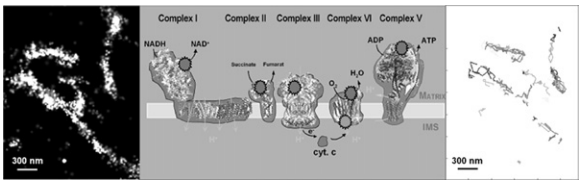
Mitochondria are cellular organelles that possess - due to their evolutionary origin - two functionally and biochemically distinct membranes. While the outer membrane encloses the organelle like a sausage casing, the inner mitochondrial membrane extrudes in the interior of mitochondrial. This cristal membrane is probably the site of oxidative phosphorylation (Gilkerson et al, 2003). Yet, the localization of respiratory complexes is not elusive to the cristal membrane (Vogel et al, 2006) as anticipated. On the other hand, cristae junctions were found to separate the two inner membrane compartments implicating a diffusion barrier for membrane proteins (Mannella et al, 2001). To reveal this discrepancy we investigated the spatio-temporal behavior of single respiratory complexes by means of superresolution imaging and single molecule tracking and found different mobilities. ATP synthase displayed the strongest confinement in the cristae when compared to other respiratory complexes. Complementary, outer membrane proteins showed an unrestricted diffusion alongside mitochondria. We thus can explain the patchy appearance of dynamic mitochondria as observed recently (Muster et al, 2010) as to be the result of hindered diffusion within the inner mitochondrial membrane.



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Focal Adhesion Formation on Cell Protrusions in 3D Shown by Modulation Tracking

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Focal adhesion formation of cells in the 2D environment has been studied in great details. However, in the natural 3D environment, focal adhesion proteins may have distinct protein dynamics, and cells may employ different mechanism for cell movement. Instead of forming filopodial or lamellipodial protrusions as seen on conventional culture dish, when embedded in collagen gel, MDA-MB-231 cells produce long protrusions that grab extra cellular matrix and guide the movement. Here we applied an optical imaging system called modulation tracking (MT) that enables the study of protein dynamics in 3D and shows the nanometer-scale resolution feature that cannot be detected using conventional confocal microscope. MT is done by moving a laser spot along the protrusion and detecting the fluorescence as the spot approaches the surface. Both fluorescent and second harmonic generation signals from collagen are simultaneously recorded. To study the cell protrusion in 3D, MDA-MB-231 cells were transfected with fluorescent focal adhesion-related proteins (paxillin, FAK & vinculin) cultured in type I collagen gel. We showed reconstructed fine 3D structures where focal adhesion proteins along the protrusion appear to grab or wrap around the collagen fibers. We also obtained direct evidence of matrix displacement by cell protrusion attached to the collagen fiber using 3D time-lapse imaging. RICS method was then applied in 3D to study the diffusion of proteins at and near the adhesions. Focal adhesion proteins have a slow (0.1um2/s) diffusion at the focal adhesions and fast diffusion in the cytosolic regions. MT is a powerful high spatial resolution technique that enables the study of cells in the 3D environment. With more details of cell protrusion and cellular movement revealed by this technique, we expect to have a more thorough and physiologically relevant view of cell migration.

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Structural Fluctuation and Catalytic Function of F_1-ATPase

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Protein dynamics has been recognized as a factor responsible for the powerful catalysis. Over the decades, many experimental and theoretical studies have provided the evidence that dynamic fluctuation positively contributed to their catalysis; however, how the fluctuation affect which elementary step of catalysis remains poorly understood. Recently, in order to elucidate at elementary-step resolution, various proteins are observed at the single-molecule level. Among them F_1-ATPase (F_1) is the excellent system in which we can not only measure almost all elementary-steps of the catalysis, but also apply the indicate amount of the viscous load on its conformational change. Accordingly, we tried to characterize the effect of the dynamic fluctuation on the catalysis of F_1. F_1 is the rotary molecular motor, which couples ATP hydrolysis to rotary motion. F_1 consists of $\alpha_3\beta_3\gamma$ subunits, in which $\alpha_3\beta_3$ and γ compose the stator ring and the rotary shaft. In order to visualize the rotary motion, we attached the rotary probe on γ. The size of the probe was much bigger than that of γ, and therefore, the actual viscous drag of γ was increased, in other word, thermal fluctuation became slower. Then, we observed the rotary motion by using the various sizes of probes (f=40~500nm), and studied the effect of the dynamics of the attached probes on the rate of ATP hydrolysis at elementary-step resolution. The rates of ATP binding and cleavage slightly depended on the viscous drag; on the other hand, P_i releasing rate was drastically accelerated as the viscous drag was decreased. Furthermore, we build the simulation model based on the previous study, and well reproduced and confirmed the experimental result computationally. This study is the achievement in which the effect of the thermal fluctuation on the catalysis is quantitatively evaluated at elementary-step resolution.

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Evaluation of the Solvent Slaving Concept in Myoglobins Through the Use of Glassy and Sol-Gel Matrices

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Functionally important protein dynamics come in a myriad of amplitudes and time scales which makes systematic studies challenging. The Solvent Slaving Model (SSM) orders protein dynamics based on the premise that protein motions are slaved to solvent motions with there being a hierarchy based on which solvent motions the specific protein dynamics are slaved (1, 2). The Protein Dynamic State Model (PDSM) is an extension of the SSM that defines dynamic states based on the temporal window during which specific solvent slaved dynamics are active(3, 4). Both models have yet to be tested systematically. In the present study, the activation energies (Ea's) for several functionally important dynamics are determined for a series of mutants of myoglobin as a function of different solvent matrices chosen to permit manipulation of specific solvent dynamics (e.g α and β relaxations). The matrices used include both trehalose glasses (β relaxations active but no α relaxations) and thin sol-gel films bathed in water/glycerol mixtures (both α and β relaxations active). The results show that for a diverse group of protein dynamics, including conformational averaging and side chain fluctuations and relaxations, the Ea's can be grouped into two broad categories: one with low Ea's (between 5 and 20 kJ/mol) and the other with much higher values (50-70 kJ/mol). The results expose how the time ordering for the onset of the functional influence of different dynamics is the result of the interplay between solvent dependent activation energy and the number of solvent slaved steps required to achieve the specific dynamical process.

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